they advance in age, however, no longer do. Oocyte donors with obviously excessive FOR are, therefore, unsuited to answer the question Lledo and associates claimed to have answered in their study.

Since the 2010a publication, we have further explored the relevance of ovarian FMR1 genotypes and sub-genotypes, with rather remarkable affirmation of their significance in affecting FOR and ovarian aging in general, but also for other important physiologic functions (Gleicher et al., 2010b, 2011, 2012; Weghofer et al., submitted for publication). Indeed, it now appears that the FMR1 gene not only affects ovarian function but sits at a crossroads of reproduction, autoimmunity and female cancer risks (Gleicher et al., 2010b; Weghofer et al., submitted for publication).

Conflict of interest

N.G. and D.H.B. are listed as co-inventors in pending US patents, which claim diagnostic benefits from assessing CGGn in women with DOR. None of the three authors has any other potential conflicts in regards to this publication.

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Reply: Intermediate and normal sized CGG repeat on the FMR1 gene does not negatively affect donor ovarian response

Sir,
The general population distribution of CGG repeats on the FMR1 gene (Fragile X syndrome) shows a prominent peak between 29 and 30 triple repeats (Fu et al., 1991). From this data, 30 repeats have been suggested as the switching point between positive and negative effects of the FMR1 gene product (Chen et al., 2003). Therefore, special attention could be taken into normal and intermediate range. For intermediate carriers, the main drawback to show an association between premature ovarian failure (POF) and CGG repeats is the cut-off for this range. A recent study considers intermediate sized 35–58 and concludes that intermediate sizes should not be considered a high-risk factor for POF (Bennett et al., 2010). It is more difficult is obtain a clear result and association for normal range. In the normal range (<35 repeats) milder forms of POF have recently also been reported (Gleicher et al., 2009a; Streuli et al., 2009).

The Gleicher group has been working hard in order to report an effect of normal and intermediated CGG repeats range in ovarian reserve. They have shown a direct statistical association between the number of CGG repeats in normal range and ovarian reserve, as reflected by anti-Müllerian hormone levels (Gleicher et al., 2009a).

However, until now no data have been published concerning ovarian stimulation and CGG repeats in normal range in fertile patients. To date, only one study has been published concerning ovarian stimulation and FMR1 expansion in infertility patients (Gleicher et al., 2009b). This study reported differences in gonadotrophin dosages and oocytes retrieved in patients carriers of >35 CGG repeats. The research published by Gleicher et al. (2010) considers the oocyte yield but does not include information about days of stimulation and gonadotrophin dosage. Moreover, we include in our study 204 egg donors where the Gleicher study includes 34. According to Gleicher comments in both studies the results in egg donors group agree and did not show statistically significant difference in ovarian response between FMR1 genotypes and sub-genotypes.

We proposed evaluating the ovarian stimulation in a non-confounding model with patients from an egg donation program because egg donors are young, fertile women with normal ovulation and there is minimal variability in oocyte and embryo quality. Then, from our point of view represent the best model because the effect of the age and the infertility could be diminished. And due to these facts we concluded that the FMR1 screening may not be considered
for predicting responses to ovarian stimulation in young and fertile females.

References


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**POLG mutations and age at menopause**

Sir,

We previously reported a dominantly inherited POLG mutation p.Y955C, in the polymerase domain of DNA polymerase gamma, which was associated with premature ovarian insufficiency (POI) and a complex neurological syndrome including ophthalmoplegia and parkinsonism in a three-generation pedigree from New Zealand (Pagnamenta et al., 2006). POLG encodes the catalytic subunit of DNA polymerase gamma (Poly), the only DNA polymerase able to catalyse mitochondrial DNA (mtDNA) replication. Impaired Poly function leads to the accumulation of multiple mtDNA deletions and progressive mtDNA depletion (Longley et al., 2005), resulting in impaired energy production by the oxidative phosphorylation enzymes (13 subunits of which are encoded by mtDNA) and increased reactive oxygen species (ROS)-induced tissue damage. Poly dysfunction has been linked to a range of neurological phenotypes (Copeland, 2012), associated with POI in a small number of cases (Table I), and also to male infertility (Rovio et al., 2001).

The mitochondrial theory of ageing postulates that mitochondrial dysfunction, including the accumulation of multiple mtDNA deletions and abnormal ROS production, is central to normal human ageing (Hekimi et al., 2011). A transgenic mouse model harbouring an error-prone Poly, caused by a mutation in the exonuclease domain of Polg, accumulates multiple mtDNA deletions and appears to mimic an ageing phenotype (Trifunovic et al., 2004). It is not clear whether POLG mutations could lead to premature ageing phenomena such as isolated POI in humans.

To determine whether POLG mutations may be responsible for isolated POI, we have now screened a cohort of British women with POI for p.Y955C and the three most common pathogenic mutations in POLG (p.A467T, p.W748S and p.G848S), using a combination of restriction fragment length polymorphism and Sanger sequence analysis, in order to determine whether these four mutations are major contributors to premature ovarian ageing. We did not identify any of these four POLG mutations in 57 women who presented with POI: 15 with primary amenorrhea, 42 with secondary amenorrhea (mean age of onset 23 years, range: 14–32). There was a positive family history in 14 cases. Diagnosis was based on raised FSH >20 IU/l on two occasions, and fragile X premutations had previously been excluded. POLG mutations have now been linked to POI in only 13 published cases, 12 of whom had neurological symptoms typical of mitochondrial disease (Table I). The 13th case was one of a cohort of 201 women with POI screened for the p.R953C mutation in POLG (Tong et al., 2010). The contribution of p.R953C mutation to her POI phenotype is unclear, and may have been a chance finding (Tong et al., 2010). We conclude that the POLG mutations more usually associated with neurological disease are not a common cause of isolated POI, and hypothesize that POLG mutations result in premature ovarian ageing only in the context of complex neurological disease.

Interestingly, a recent meta-analysis of genome-wide association studies searching for loci and potential candidate genes determining age of natural menopause identified POLG amongst the top 11 candidate genes (Stolk et al., 2012). The availability of high throughput next generation DNA sequencing techniques will now allow whole genome sequence analysis in large cohorts of women whose menopausal age is known, in order to establish whether genetic changes in POLG are associated with age at natural menopause. Functional analyses, including assessment of mtDNA integrity and copy number and ROS production in ageing ovarian tissue, will also be important in determining the relative contribution of Poly dysfunction to ovarian ageing. However, the results of the meta-analysis suggest that the individual contribution of Poly dysfunction to the age of natural menopause is likely to be small (Stolk et al., 2012), and that the synergistic effect of many individually small factors probably orchestrates the complex process of natural ovarian ageing.